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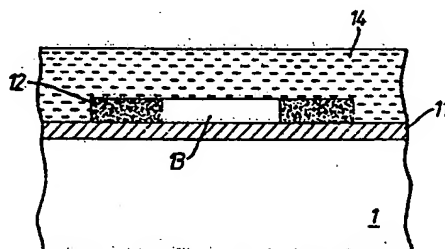
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(54) **Biosensor device**

(57) A biosensor device, comprises a pair of substantially parallel electrical conductors 12 laid on a surface 11 of electrical insulation material on a silicon substrate 1 and an immobilised reagent 13 containing an enzyme coated over the pair of conductors. Addition of an analyte to the reagent material causes a reaction varying an electrical or a thermal characteristic which is measured using the conductors 12. The measuring technique may measure surface conductance by determining AC resistance, temperature change by resistance thermometry or may determine the analyte concentration by amperometry using a DC applied potential. A reference sensor may be incorporated on the substrate. Various suitable enzymes, optionally containing mediators, and methods for immobilising the enzymes on the sensor are disclosed.



**FIG.3.**

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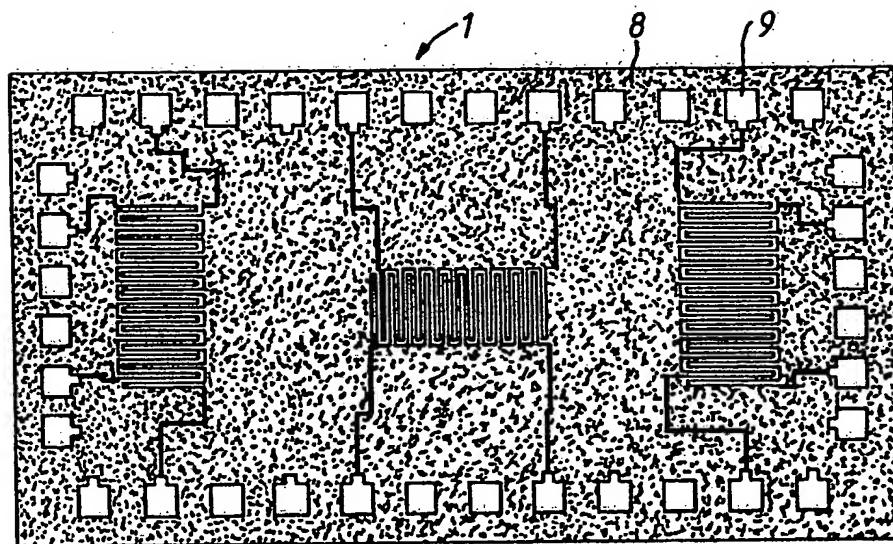
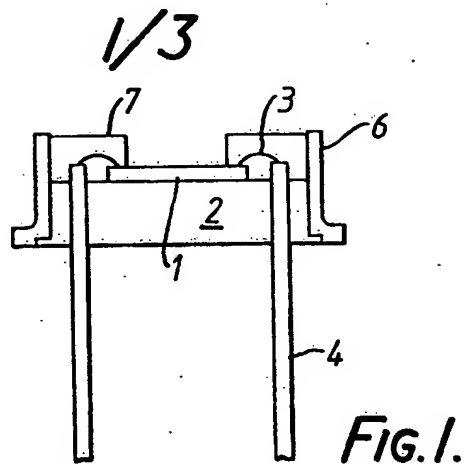


FIG. 2.

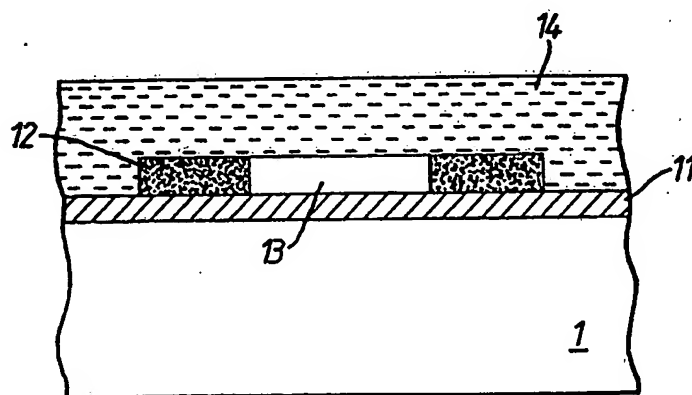


FIG. 3.

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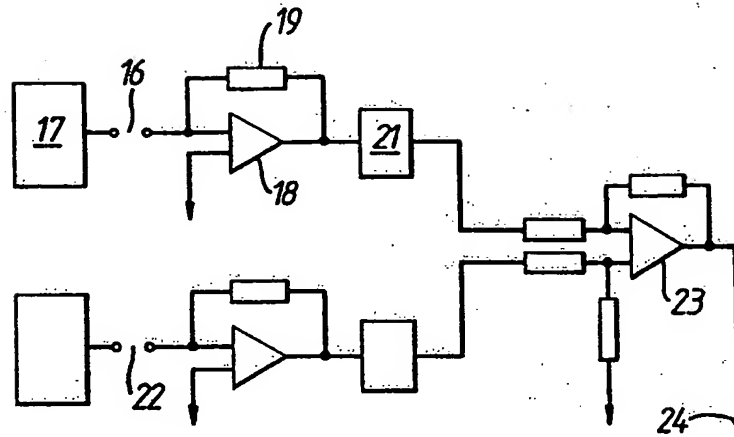


FIG. 4.

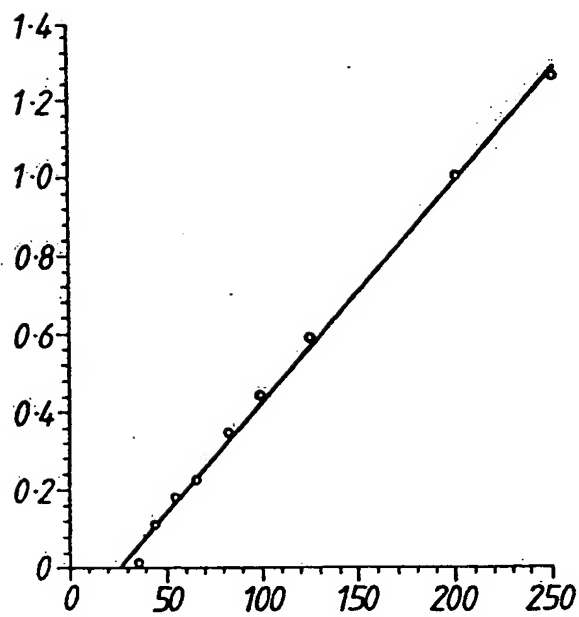


FIG. 5.

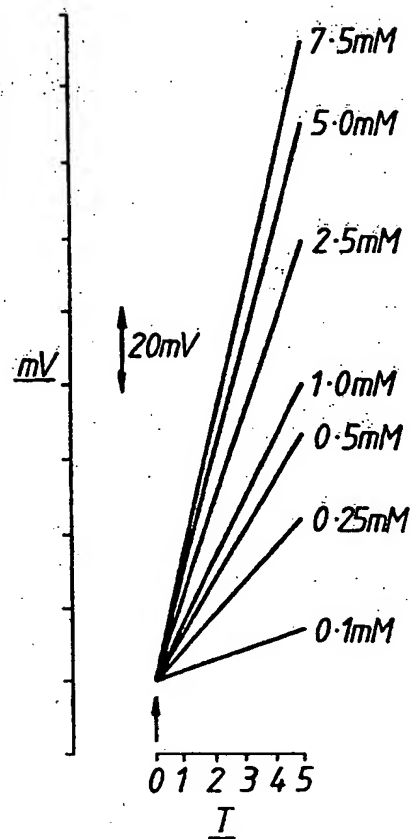


FIG. 6.

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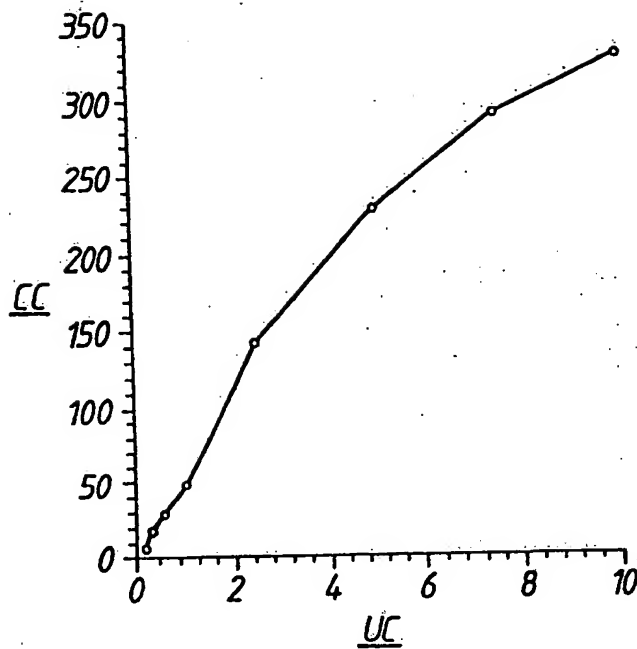


FIG. 7.

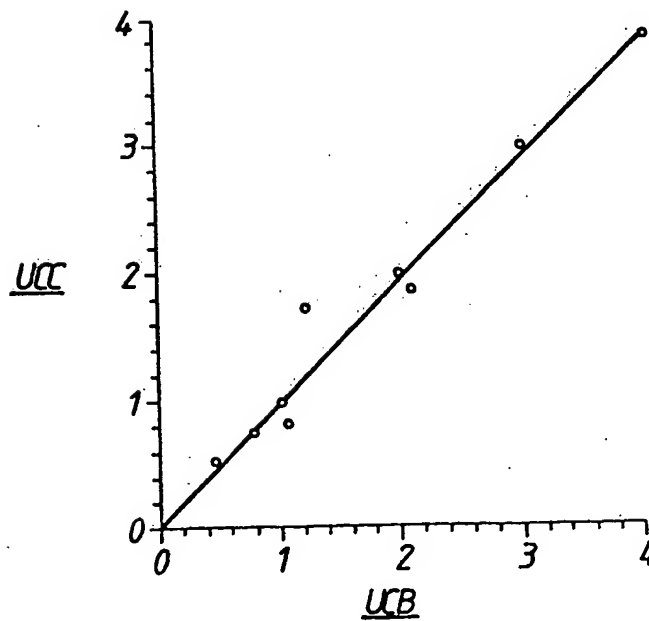


FIG. 8.

BIOSENSOR DEVICE

This invention relates to a biosensor device which can be utilised in a multi-analyte application and which can enable any one of three different measuring principles, namely surface conductance, amperometry and  
5 resistance thermometry, to be employed.

The device comprises a pair of substantially parallel electrical conductors supported on a surface of electrical insulation material. By the words 'substantially parallel' as used in this specification, it is meant to  
10 include arrangements such as a single electrode which may be folded or serpentine to give a parallel arrangement of parts of this conductor. In addition, the expression includes an arrangement of two separate electrodes which could have interdigitated parts.

15 With the conductors supported on the surface, an analyte reagent mixture may be placed thereon thus enabling an electrical property of the analyte reagent mixture to be measured and changes in that electrical or thermal property to be determined.

20 In a preferred embodiment of the biosensor device, the conductors are laid upon the surface of a silicon chip. This operation may be effected simultaneously with the fabrication, on the same chip, of ancillary circuitry to enable the electrical property measurements or changes

to be determined. Such an arrangement permits very small parameters or parameter changes to be measured without the influence of noise, the presence of which can be inherent when external electrical circuitry is employed. Further, 5 the device may include a quantity of an immobilised reagent material which is placed so as to overlie the conductors. Such a reagent material may be an enzyme which alters the conductance of an applied analyte, a redox enzyme enabling amperometric detection of a redox 10 reaction when a voltage is applied across the conductors in the presence of an analyte, or an enzyme which causes local heat changes in an analyte.

If the sensor is to be used in amperometric detection, an immobilised enzyme thereon may contain 15 electron transfer mediators to expedite the diffusion of reaction products and of electrons to the conductors.

If the sensor is to be used in resistance thermometry, the conductors may be of nickel or a metal of similar high temperature coefficient of resistance or a 20 semiconductor material.

The reagent material may form part of an enzyme/analyte system applied to the sensor to enable measurements to be made.

Advantageously, a further pair of conductors is 25 fabricated in the same way and preferably at the same time, to act, in use, as a control means whereby the

effects of temperature and other variations may be eliminated. A description of preferred embodiments of biosensor devices according to the present invention will

now be given with reference to the accompanying  
5 drawings, in which:-

Figure 1 is a diagrammatic cross-section of a biosensor device according to the present invention,

Figure 2 is a plan view of a part of the device, showing the arrangement of electrical conductors,

10 Figure 3 is a diagrammatic cross-section of part of the sensor device, in use,

Figure 4 is an electrical circuit diagram of the component arrangement required to make the necessary electrical property measurements,

15 Figure 5 is a graph of a calibration curve for the electrical circuit,

Figure 6 is a graph illustrating the variation of surface conductance with time for a soluble urease/urea system in a device according to the present invention,

20 Figure 7 is a graph illustrating the performance of the surface conductance sensor for an immobilised urease/urea system, and

Figure 8 is a graph illustrating a comparison of urea concentrations in human serum as determined by a hospital  
25 laboratory testing procedure and by the microconductimetric biosensor of the invention.

The fabrication of the preferred devices according to the invention involves the following main operations on a silicon wafer: thermal oxidation; metallisation; photolithography and etching; sawing; chip bonding; wire bonding and encapsulation; and deposition of the required enzyme/immobilisation material. Some of these operations make use of existing semiconductor device fabrication techniques. The details are given below:-

- 10 (1) A silicon wafer is thermally oxidised in a furnace using a standard oxidation process to provide a layer approximately 550nm in thickness of silicon dioxide ( $\text{SiO}_2$ ) on its surface.
- 15 (2) A multimetal coating is next deposited onto the oxidised silicon surface using a standard sputter deposition process or thermal evaporation under vacuum. Four alternative examples of metallisation scheme employed are:  
titanium/gold, titanium/platinum/gold, chromium/gold  
20 and chromium/platinum/gold. The thickness of each of the metals titanium, platinum and chromium was usually around 100nm whereas that of gold varied between 1,000nm to 3,300nm depending upon the particular application.
- 25 (3) The required substantially parallel metal conductor tracks/patterns 1,200nm to 3,500nm thick and 5,000nm



wide are produced by using the following photolithographic process(es) and metal etching.

Process (a): (Resist material thickness about 1,000nm)

5 A small quantity of 3-mercaptopropyl-trimethoxy-silane liquid (MPTS: sold by Fluke AG, Switzerland) was spread on the metallised wafer and it was then spun on a motor-driven turntable at a rate of 5,000 rpm for forty seconds. The resulting  
10 layer which was formed acted as a surface adhesion promoter. A layer of a positive photolithographic resist material AZ1350H (Hoechst, FR Germany) was next spread on the wafer, it was spun at 5,000 rpm for forty seconds and then prebaked in an oven at a  
15 temperature of 90°C for twentyfive minutes. The wafer was aligned with the mask (one repeat pattern of which is depicted in Figure 2) on a MJB-21 model mask aligner machine (Karl Suss, FR Germany) and exposed to ultraviolet radiation for eightyfour  
20 seconds. Photographic development took place in "Microposit" developer (Hoechst, FR Germany) (at a concentration of 1:1 in dilution with water) for thirtyfive seconds and the wafer was then postbaked at 90°C for twentyfive minutes.

Process (b): (Resist material thickness about  
6,300nm)

A small quantity of the surface adhesion promoter  
MPTS liquid was spread on the metallised wafer and  
5 spun at 4,000 rpm for sixty seconds. Next, a layer of  
a positive  
resist material AZ4620 (Hoechst, FR Germany) was  
spread on the wafer, it was spun at 5,000 rpm for  
sixty seconds and then prebaked at 90°C for thirty  
10 minutes. The wafer was aligned, as under process  
(a), exposed to ultraviolet radiation for 225  
seconds, developed in a photographic developer K400  
(Hoechst, FR Germany) (at a concentration of 1:3 in  
dilution with water) and then postbaked at 90°C for  
15 thirty minutes.

The required metal patterns were produced by etching  
metals through developed resist patterns either by  
dry etching techniques such as ion-beam milling for  
removing all the metals (gold, platinum, titanium,  
20 and chromium) or by a combination of standard wet  
etching (of gold, titanium and chromium) and dry  
etching (of platinum) techniques. It is preferred  
to employ resist deposition process (a) for wet  
etching whilst process (b) is preferred for dry  
25 etching.

(4) The remaining resist material was subsequently removed by dissolving in acetone or standard AZ resist remover (Hoechst, FR Germany). The silicon wafer was finally subjected to a washing sequence involving deionised water, acetone, methanol and isopropyl alcohol. The wafer was then sawed by a standard process to produce discrete silicon chips each one containing one to three surface devices.

10

(5) As depicted in Figure 1, the chip was mounted on and bonded to a standard twelve pin T05 header package 2. The bonding was effected using an epoxy resin bonding material or in a different embodiment a gold/tin (80:20) eutectic solder composition. When solder is employed for bonding, it is preferable to metallise the rear surface of the silicon chip; this improves the adhesion to the header. The connections to the devices were made by the thermocompression wire bonding technique (using lengths of gold wire 3 of thickness about 25,000nm) secured onto the device pads and pin-heads of the pins 4 of the package 2. An open-ended can 6 was welded to the header. The electrical connections to the devices were then

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20

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encapsulated in a body of epoxy resin 7 leaving exposed an appropriate number of the parallel conductor networks.

Figure 2 shows on a greatly enlarged scale the microcircuit surface. In this embodiment, the discrete silicon chip 1 carried three surface devices 8 each of which comprised a pair of substantially parallel electrical conductors. It will be seen that each device 8 is formed of two long serpentine electrical conductors the two ends of each conductor terminating in a contact pad 9 located on the outer edge of the chip 1. The two conductors of each device 8 are serpentine together so as to provide a portion of each conductor which is in a substantially parallel relationship with a similar portion of the other conductor.

It will be clear that in a different embodiment it will be possible to position the electrical conductors in some alternative manner which will still give the required parallel relationship between parts of the conductor(s). One such alternative construction would be to have an interdigitated arrangement. A single electrical conductor which was laid down in a serpentine arrangement could also be used since the necessary pair of parallel conductors could be formed from different parts of the same metal strip.

Figure 3 shows in a further enlargement a cross-sectional view of part of the chip 1 supporting the electrical conductor areas.

In this view, the silicon substrate material constituted by the chip 1 has had a surface layer 11 of silicon dioxide formed on its uppersurface. The surface layer 11 is of course an electrical insulation material and this serves to support the metal electrical conductor areas 12. The view in cross-section shows two conductor areas 12 and a volume of an immobilised enzyme material 13 has been placed between these two areas. The area of electrodes and enzyme material 13 is then covered over with a quantity of an analyte 14.

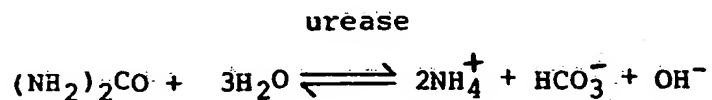
#### I SURFACE CONDUCTANCE DEVICE

The miniaturised surface conductance device offers potential for the measurement of many biological substrates when their complementary enzymes are immobilised across the surface of the electrical conductor networks. Enzymes are chosen which by their specific catalytic action generate or consume ionic species and thereby lead to a change in solution conductance. The procedure is generally applicable to deaminases, amidases,

oxidases, proteases, nucleases, other hydrolases, decarboxylases, kinases, lipases, phosphatases and many other enzymes.

### Urea Biosensor

- 5        For example, the enzyme urease catalytically cleaves a neutral substrate urea into positively charged ammonium ions and negatively charged bicarbonate and hydroxyl ions:



- 10      Thus, urease immobilised to the surface of the parallel conductor pattern would enable the presence of urea in an analyte to be detected by observing conductance changes measured across the two tracks of the above pattern (Figure 3) as the enzyme cleaves the urea.

### 15      Construction and Operation

- The electrical circuit about to be described was designed to make use of conventional alternating current conductimetric monitoring techniques in order to reduce Faradaic processes, double-layer charging and
- 20      concentration polarisation at the microelectrode surface.

The conductance biosensor was normally operated in a

dual cell configuration comprising a reference pair and a sample pair of the parallel electrical conductors. Accordingly, therefore, the circuit diagram as depicted in Figure 4 shows connections for a sample conductance cell 16 which receives an a.c. signal from a monolithic integrated circuit waveform generator 17 (No RS8039CC from RS Components Ltd., Corby, Northants, UK). An output terminal of the cell 16 is connected to a high input impedance FET operational amplifier 18 (RS Components, CA3140) and this has a fixed feedback resistor 19 (10 megohms plus or minus 1%) with the conductance cell forming an input resistor.

An output signal from the amplifier is taken to an RMS-DC converter integrated circuit 21 (RS Components Ltd, AD563A) such that the output voltage could be monitored on a chart recorder (Gould BS-272) and/or a digital multimeter (Hewlett Packard 3468A).

The circuit as just described would be suitable for operation in single cell mode with the output voltage from the RMS-DC converter circuit 21 being taken directly to the chart recorder. As already mentioned, however, a dual cell configuration is preferred and thus the circuit of Figure 4 includes the sample conductance cell 16 together with a reference cell 22. The full circuit uses two

identical sine wave generator 17/inverting operational  
amplifier 18 units with the outputs from the two reference  
and sample RMS-DC converters 21 being taken to a  
conventional differential amplifier 23 and thence to a  
5 chart recorder/digital multimeter output terminal 24.

The biosensor cells were temperature equilibrated to  
30 plus or minus 0.1°C by means of a glass water jacket  
connected to a Julabo 20B heating/circulating water bath.

In operation of the circuit of Figure 4, the  
10 integrated circuit waveform generators 17 apply a low  
distortion sine wave of frequency 1kHz and amplitude zero  
volts plus or minus 5mV to the sample and reference cells.  
The inverting operational amplifiers 18 provide gain and  
serve to amplify the responses from the cells. The  
15 output signals from the amplifiers 18 are converted into  
DC voltages via the integrated circuit RMS-DC converters  
21.

The instrument was calibrated by relating the DC  
output of the RMS-DC converter 21 to reciprocal ohms  
20 (Siemens) by connecting low temperature coefficient about  
1% tolerance, 0.25W metal-film type resistors (having  
values between 56k and 10megohms) across the sample pair  
of microelectrodes in order to simulate solution  
conductance changes. This calibration procedure  
25 permitted the output voltage of the conductance biosensor  
to be related to the S.I.units (Siemens) measurement for  
conductance.



Figure 5 is a graph of a calibration curve for the electrical circuit where the horizontal axis shows Conductance (in microSiemens) against Output Voltage (in volts) on the vertical axis. The output voltage from the RMS-DC converter 21 is seen to be linearly related to the calibration conductance with a correlation coefficient of 0.998 as deduced by linear regression analysis. This calibration curve suggests that the output voltage from the RMS-DC converter 21 will be linearly related to the change in ionic strength generated during the initial period of an enzyme-catalysed reaction, provided that the basal ionic strength of the buffered solution is low enough to permit an adequate signal to background noise ratio. For this reason, a buffer of low intrinsic conductance, imidazole, was used throughout for the measurements.

#### Enzyme Immobilisation

Urease (urea amidohydrolase, EC 3.5.1.5) was immobilised over the sample network by carefully adding 10 microlitres of a mixture of equal volumes of enzyme (100 mg/ml), bovine serum albumin (100mg/ml) and glutaraldehyde (2.5% by volume) solution, all made up in 5mM imidazole-HCl buffer, pH 7.5. An enzyme albumin gel formed after nine or ten minutes at 20°C and it was then

washed exhaustively with 5mM imidazole HCl buffer, pH 7.5.

#### Sample Measurement Protocol

The microelectronic conductance biosensor was initially calibrated against known concentrations of urea. Imidazole-HCl buffer (5mM pH 7.5) (180 microlitres) was added to the device equilibrated to 30°C, whence, once a stable baseline had been achieved after a time period of from thirty to sixty seconds, a known concentration of urea (0-100mM) (20 microlitres) was added. The output voltage from the differential amplifier 23 was monitored over a three to four minute time period to ensure linearity.

A modified buffer solution was used to monitor the levels of urea in human plasma. The instrument was calibrated in 5mM imidazole-HCl buffer, pH 7.5 containing 4mM NaCl and 1.6g/l human serum albumin in order to simulate the background conductance anticipated from 25-fold diluted human serum. Serum samples of known urea concentration were obtained from a hospital laboratory, diluted 25-fold in the imidazole/NaCl/albumin buffer and assayed for urea in the conductance biosensor. Unknown clinical samples were interpolated from a calibration curve for urea produced in the same buffer.

The determination of the electrical conductivity of solutions depends on the accurate measurement of the electrical resistance between two electrodes of defined geometry immersed in the conducting solution. In the differential microelectronic conductance biosensor described here, the device comprises identical pairs of multimetal electrodes fabricated on a silicon substrate in a planar configuration so that these requirements can be achieved.

#### 10 Soluble Urease/ Urea System

Initially characterisation of the conductance response of the simplified soluble urease/urea system was obtained. Typical responses of the urease-loaded conductance biosensor operating in single cell mode to several concentrations of urea in 5mM imidazole-HCl buffer pH 7.5 containing 10 micrograms per millilitre of urease at 30°C are shown in Figure 6.

Figure 6 is a graph which shows on a vertical axis the cell conductance change in mV as measured on an arbitrary scale with each of the marked units on the axis being equal to about 20mV. The horizontal axis measures elapsed time T (in minutes) and an addition of urea is made to the cell at the time, T equal to zero minutes. The resulting straight line responses indicate the change

in conductance taking place in response to the addition of various quantities of urea ranging from 7.5 mM to 0.1 mM.

The responses demonstrate the stability of the baseline and, after the addition of the urea, the linearity of the output of the instrument over a five minute period. The responses to any given concentration of urea were found to be reproducible to within about 1%; for example, at 2.5mM urea, the output voltage of the RMS-DC converter 21 was 24.0 plus or minus 0.21 mV.  $\text{min}^{-1}$ , whilst at 7.5 mM urea the output was 34.8 plus or minus 0.39 mV.  $\text{min}^{-1}$ .

#### Immobilised Urease System

A quantity of urease (from Jack Bean, Sigma Chemical Co.) was immobilised over the metal network by forming a cross-linked enzyme-albumin membrane with glutaraldehyde.

The output response of the cell to this system is depicted in Figure 7 which is a calibration curve for urea in 5mM imidazole-HCl buffer, pH 7.5 at 30°C in the differential mode conductimeter with immobilised urease. Figure 7 shows on the vertical axis the change in cell conductance (CC) measured in  $\text{mV. min}^{-1}$  which is thus the output response of the differential amplifier 23. On the horizontal axis, the graph shows urea concentration (UC) in the range of 0.1 to 10mM. As in the case of soluble

urease, linear responses of duration up to five minutes were obtained at all urea concentrations.

The immobilised urease conductometric biosensor also responded to urea present in serum samples. The instrument was calibrated in 5mM imidazole-HCl buffer, pH 7.5 containing 4mM NaCl and  $1.6\text{g.l}^{-1}$  human serum albumin in order to simulate the background conductance anticipated from a 25-fold diluted human serum sample. Serum samples of known urea concentrations were obtained from a hospital laboratory, diluted 25-fold in the imidazole/NaCl/albumin buffer and assayed for urea in the conductance biosensor.

The results are depicted in Figure 8.

Figure 8 is a graph showing a comparison of urea concentrations as tested by the hospital laboratory and by the conductimetric biosensor. On the vertical axis, the graph shows urea concentration (UCC) in mM as measured by the clinical testing method. On the horizontal axis, the urea concentration (UCB) of the samples as measured by the conductimetric biosensor is given.

Figure 8 shows that there was a linear relationship (correlation coefficient greater than 0.99) between the urea concentrations determined with the microelectronic device and those obtained from the hospital laboratory.

In all the examples, the sensor was normally operated in the differential mode. An enzyme-loaded

albumin membrane was cast over the 'sample' pair of electrodes but not over the 'reference' pair, such that subtraction of the amplified signals emanating from the respective RMS-DC converters continuously corrects for any background signal changes. This simple expedient

5 circumvents many of the problems that can be associated with non-specific variations in basal conductivity of the buffers and biological fluids in which measurements are being made.

10 It is expected that this miniaturised device and associated instrumentation will find application in the analysis of urea for in vitro blood analysis, in renal surgery and in dialysis monitoring, for example.

#### 11. AMPEROMETRIC DEVICE

15 The amperometric method of detection using a device according to the invention essentially relies on the imposition of a constant voltage across the two parallel conductors in the test environment. A diffusion current which can be measured flows through the system. This  
20 current is the result of an electrode reaction taking place under the applied voltage due to electron transfer, and is proportional to the concentration of the test species. The parallel conductors can thus be exploited as base electrodes for amperometric devices.

The electrode reaction may involve directly the

species being detected or, indirectly, one of the products of an enzyme catalysed reaction. In the latter case, to effect rapid (diffusion-controlled) electrochemical reaction, it is sometimes essential to employ suitable electron transfer mediators to deliver electrons from the products to the electrode. The mediators may be provided in the immobilised enzyme membrane or in the buffer solution forming the analyte. The voltage across the electrodes must be sufficient to cause the desired reaction, for example, a redox reaction, to take place. It must not be so high that it causes undesired reactions to occur at the electrodes. Those skilled in the art will appreciate that this requires the operation of for example an enzyme electrode (immobilised enzyme on the parallel conductor network) in a potentiostatic mode against a suitable reference electrode such as a saturated calomel electrode. The test species may be an enzyme substrate or product, cofactor or even an oxidation state of the enzyme itself. A number of redox enzymes can be used but particularly those active on substrates such as monosaccharides (glucose, galactose), fatty acids, hydroxy acids, amino acids, purines, pyrimidines, aldehydes, thiols, phenols and steroids. The mediators can be physiological or non-physiological. The latter offer a variety of organic, inorganic and metallo-organic redox species which can be immobilised on to the electrode

surface or within the enzyme layer. By the judicious choice of mediators having specific values of redox potentials, it is possible to overcome problems of interference due to other competing electrode reactions, for example electroactive species that are present in many authentic biological analytes.

### 111. RESISTANCE THERMOMETER DEVICE

The use of the biosensor device according to the invention in this manner is based on the phenomenon of the temperature coefficient of resistance of metals or semiconductor materials. The parallel conductors with a biological layer thereover can be exploited to monitor absolute temperature by measuring resistance changes down the lengths of the conductor tracks as a function of temperature. Nickel conductors are preferably employed for this purpose since this metal has a particularly high temperature coefficient of resistance. The catalytic action of enzymes, organelles or whole cells may generate local heat changes, either directly or via protonation of buffer salts. Such changes thus permit the present devices to be used for monitoring the concentrations of the (biological) substrates of such catalytic action by measuring the differential thermal change. This approach could be used to monitor a wide range of clinical, veterinary, agricultural, pollution or fermentation analytes.



Thus, a biosensor device containing a number of parallel conductor paths forming resistance network patterns can be exploited as a miniaturised multi-analyte sensor. Potential advantages of this type of multisensor, in addition to simultaneous analysis of more than one analyte, include elimination of temperature effects on measurements, measurement of temperature changes and additional confirmation of results obtained by two independent techniques.

10 It will be appreciated that the invention has been described in detail with reference to particular devices and their uses, but the invention is not to be considered as being so limited and variations within the scope of this disclosure are possible.

15 For instance, the integrated circuit chip has been described as being accommodated in a standard twelve pin T05 header package, but this type of package is not of course essential and any suitable single or multilayer chip carrier could alternatively be used.

20

CLAIMS

1. A biosensor device comprising a pair of substantially parallel electrical conductors supported on a surface of electrical insulation material, and extending thereover an immobilised reagent material whereby, on exposing the reagent material to an analyte, variations in an electrical or thermal characteristic of the analyte/reagent may be measured.
2. A device as claimed in Claim 1, wherein the insulation surface is a layer of silicon dioxide on the surface of a silicon substrate and the conductors are laid thereon by an integrated circuit fabrication technique.
3. A device as claimed in Claim 1 or Claim 2, comprising a plurality of the pairs of conductors at least one of which has a quantity of an immobilised reagent material extending thereover and the or some of the remaining pairs are exposed so that, when an analyte is placed thereon, a basic electrical characteristic of the analyte alone can be measured.
4. A device as claimed in Claim 1 or 2, wherein a plurality of the pairs of conductors is provided and at least one of the pairs of conductors is then covered with a masking material.

5. A device as claimed in Claim 2 or any claim appendent thereto, wherein electrical circuitry, ancillary to the measurement of the electrical characteristic or characteristics to be measured, is fabricated on the same silicon substrate.

6. A biosensor device comprising a silicon substrate, a layer of silicon dioxide thereon forming a surface of an electrical insulation material, at least one pair of substantially parallel electrical conductors on the insulation surface, the or at least one of the pairs presenting an exposed surface whereon an immobilised reagent material and thereafter an analyte, or a reagent material and an analyte mixture may be placed, encapsulation means for protecting the remainder of the silicon substrate, and electrical connection means for enabling an electrical characteristic of the reagent and analyte to be measured.

7. A device as claimed in any preceding claim, wherein the reagent material is an enzyme.

8. A device as claimed in Claim 7, further including an electron transfer mediator in the reagent material.

9. A device as claimed in any preceding claim, wherein

the electrical characteristic to be measured is surface conductance of the analyte/reagent mixture.

10. A device as claimed in any one of Claims 1 to 8, wherein the or each pair of conductors is formed of nickel, nickel-chromium or a semiconductor material and the electrical characteristic to be measured is that of resistance variation with temperature.

11. A method of fabricating a biosensor device, comprising the steps of coating a silicon substrate with a layer of silicon dioxide to form a surface of electrical insulation material thereon, laying at least one pair of substantially parallel electrical conductors on the insulation surface, encapsulating the device so as to leave at least one of the pairs exposed, providing a quantity of an immobilised reagent material over the or one of the exposed pairs, and forming electrical connections to the or each of the pairs so that, in use, an electrical characteristic of that pair with the immobilised reagent material thereover can be measured.

12. A method as claimed in Claim 11, wherein the immobilized reagent material includes an enzyme.

13. A biosensor device, substantially as hereinbefore described with reference to and as illustrated in

any one of Figures 1 to 4 of the accompanying drawings.

14. A method of fabricating a biosensor device substantially as hereinbefore described.